(r=.20). Baseline $KOOS_4$ scores were also related to all 5 year KOOS subscores (all outcomes p<0.01, r=.23 to r=.29) (Table 1).

Conclusions: Cartilage injury at baseline was related to worse patient reported 5 year outcome. Baseline subchondral fracture and meniscus injury were not related to 5 year outcome, however, receiving medial meniscus surgery was related to worse pain, QOL and knee confidence at 5 year follow-up. In comparison, lateral meniscus surgery was not related to 5 year outcome. Undergoing ACLR (early or delayed) was associated with more knee symptoms at 5 years and treatment with rehabilitation alone resulted in worse knee confidence. More joint pain, symptoms, poorer physical function and worse QOL in the acute phase of ACL injury, were related to worse 5 year outcome, irrespective of intervention.

Table 1. Results of exploratory analyses (n=118)

Predictor	KOOS Symptoms	KOOS Pain	KOOS Sport/Rec	KOOS QOL	Method	Knee confidence	Method
Injury related:							
Cartilage injury (yes/no)	**p=0.006** U=924	**p=0.043** U=1051	**p=0.049** U=1053	**p=0.049** U=1052	MU	p=0.494 χ^2=4.68	Chi²
Meniscus injury (yes/no)	p=0.203 U=1499	p=0.053 U=1385	p=0.092 U=1423	p=0.291 U=1538	MU	p=0.899 χ^2=0.02	Chi²
Impression fracture (yes/no)	p=0.294 U=1320	p=0.542 U=1397	p=0.636 U=1418	p=0.406 U=1356	MU	p=0.991 χ^2=0.00	Chi²
Treatment related:							
Medial meniscus surgery(yes/no)	p=0.086 U=1323	**p=0.017** U=1211	p=0.283 U=1437	**p=0.023** U=1222	MU	**p=0.013** χ^2=6.15	Chi²
Lateral meniscus surgery (yes/no)	p=0.226 U=1348	p=0.218 U=1349	p=0.185 U=1328	p=0.690 U=1489	MU	p=0.696 χ^2=0.15	Chi²
ACLR (yes/no)	**p=0.014** U=901	p=0.938 U=1279	p=0.261 U=1112	p=0.357 U=1144	MU	**p=0.003** χ^2=8.97	Chi²
Baseline PROs:							
$KOOS_4$ baseline	**p=0.001** r=.29	**p=0.006** r=.25	**p=0.003** r=.27	**p=0.013** r=.23	SP	**p=0.033** χ^2=4.54	KW
SF-36 PCS baseline	**p=0.005** r=.26	**p<0.001** r=.39	**p=0.002** r=.28	**p=0.001** r=.29	SP	**p=0.016** χ^2=5.81	KW
SF-36 MCS baseline	p=0.130 r=.14	**p=0.032** r=.20	p=0.338 r=.09	p=0.180 r=.13	SP	p=0.425 χ^2=0.64	KW

Knee confidence: trouble with knee confidence, none-to-mild vs. moderate-to-extreme; KOOS: Knee Injury and Osteoarthritis Outcome Score; QOL: quality of life; Method: statistical method for p value, significant results p<0.05 are highlighted in bold; ACLR: Anterior Cruciate Ligament Reconstruction; $KOOS_4$: an aggregate score from KOOS pain, symptoms, sport/rec, and QOL subscales; SF-36 PCS: SF-36 physical component score; SF-36 MCS: SF-36 mental component score; MU: Mann-Whitney U Test (U); SP: Spearman's rho (r); KW: Kruskal-Wallis ANOVA (χ^2); Chi² (χ^2)

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MICRORNA-33A REGULATES CHOLESTEROL SYNTHESIS AND CHOLESTEROL EFFLUX RELATED GENES IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: Several studies have shown that osteoarthritis (OA) is strongly associated to metabolic related disorders, highlighting OA as the fifth component of the Metabolic Syndrome (MetS). Based on our previous findings on dysregulation of cholesterol homeostasis in OA, we were prompted to investigate whether microRNA-33a (miR-33a), one of the master regulators of cholesterol and fatty acid metabolism, plays a key role in osteoarthritis' pathogenesis.

Methods: Articular cartilage samples were obtained from 14 patients with primary osteoarthritis undergoing total knee replacement surgery. Normal cartilage was obtained from 9 individuals undergoing fracture repair surgery with no history of joint disease. Bioinformatics was used to identify miR-33a target genes. MiR-33a and Sterol Regulatory Element Binding Protein 2 (SREBP-2) expression levels were investigated using real-time PCR and their expression was also assessed after treatment with Transforming Growth Factor-beta (TGF-beta) in cultured chondrocytes. Investigation of Akt phosphorylation after treatment with both TGF-beta and miR-33a inhibitor was assessed by western blot analysis. Furthermore, we evaluated the effect of miR-33a mimic and miR-33a inhibitor on cholesterol efflux related genes, ATP-binding-cassette transporter A1 (ABCA1), Apolipoprotein A1 (ApoA1), liver X receptors (LXRalpha and LXRbeta), as well as on matrix metalloproteinase-13 (MMP-13) using real time PCR assay.

Results: We found that the expression of miR-33a and its host gene SREBP-2 were significantly elevated in OA chondrocytes compared to normal. We also showed that treatment of cultured chondrocytes with TGF-beta resulted in increased expression of both miR-33a and SREBP-2, while the TGF-beta-induced Akt phosphorylation was suppressed by inhibition of miR-33a. Moreover, we demonstrated that treatment of normal chondrocytes with miR-33a resulted in ABCA1 and ApoA1 significantly reduced mRNA expression levels and significantly elevated MMP-13 expression levels, promoting the OA phenotype, while miR-33a's suppressive effect was reversed using its inhibitor.

Conclusions: Our findings suggest, for the first time to our knowledge, that miR-33a regulates cholesterol synthesis through the TGF-beta/Akt/SREBP-2 pathway, and cholesterol efflux related genes ABCA1 and ApoA1 in OA chondrocytes, pointing to its identification as a novel target for ameliorating the OA phenotype.

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THE ROLE OF MICRORNA-140 IN OSTEOARTHRITIS

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Purpose: The purpose of this study was to determine the role of microRNA-140 in an *in vitro* model of osteoarthritis (OA).

Methods: Human articular chondrocytes (ACs) and synovial fibroblasts (SFs) were isolated from patients undergoing total knee replacement and anterior cruciate ligament surgery, respectively. Addition of recombinant IL1B to cell cultures was used as an *in vitro* model of OA. All patients provided written, informed consent. Electroporation was used to overexpress microRNA-140. Real-time PCR was used to quantify mRNA levels and genome-scale proteome quantification was performed using mass spectrometry. ELISA and western blotting were used to validate results from the proteome analysis.

Results: IL1B-stimulation of ACs resulted in upregulation and downregulation of 200 and 160 proteins, respectively. The upregulated proteins included several pro-inflammatory molecules such as IL1B, IL6, IL8 and the matrix degradation molecules MMP3 and MMP13. All these proteins are known to be involved in the pathogenesis of OA.

When microRNA-140 was overexpressed in IL1B-stimulated ACs 100 and 90 proteins were downregulated and upregulated, respectively. The downregulated proteins included IL1B, IL6, IL8, proteins involved in NF-kappaB signaling and other proteins that are associated with OA. However, MMP3 and MMP13 were not affected. ACAN, CSGALNACT1 (involved in chondroitin sulfate biosynthesis), proteins involved in hyaluronan synthesis/binding and inhibitors of NF-kappaB signaling were among the proteins that were upregulated by miR-140. The remaining proteins were mainly associated with cell cycle and metabolism.

Conclusions: Addition of low levels of IL1B to cultures of ACs or SFs constitute useful *in vitro* models for the pathogenesis of OA. In this study we show that microRNA-140 protects against OA by inhibiting key inflammatory molecules and stimulating synthesis of important cartilage molecules. Delivery of microRNA-140 into OA joints may represent a viable treatment strategy.

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MICRORNA-455 TARGETS SIRT1

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Purpose: MicroRNAs (miRNAs) have emerged as a new class of gene expression regulators that are important in both normal cartilage physiology and pathology. MicroRNAs are 20-24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. A single miRNA can regulate multiple mRNAs via binding to sequences in the 3′UTR either promoting mRNA decay or repressing translation. We have previously identified several microRNAs that are regulated during chondrogenesis and in osteoarthritic cartilage, including microRNA-455, a key Sox9-responsive microRNA.

The aim of this study was to elucidate further the role of miR-455 in cartilage and OA by identifying direct targets of miR-455 in chondrocytes. In this study we have shown that miR-455 directly targets a number of genes including Sirtuin 1 (Sirt1). Sirt1 is a NAD+ dependent protein deacetylase implicated in aging and age-related diseases, including OA. Previous studies have shown that Sirt1 promotes chondrocyte survival and is down-regulated in OA cartilage. Mice deficient for Sirt1 develop premature OA like changes. Here we show that miR-455 directly targets Sirt1 and can regulate IL-1β signalling through NFκB, potentially regulating acetylation of Sirt1 target proteins such as NFκB/p65.

Methods: To identify potential targets of miR-455 we performed whole genome mRNA and miRNA array analysis in the ATDC5 cell model of chondrogenesis and mRNA array in primary chondrocytes following overexpression or inhibition of miR-455. Whole genome mRNA microarray used the Illumina Human HT-12 v4 Expression BeadChip; miRNA microarray used the Exiqon miRCURY LNA Array. We further analysed the data to identify regulated genes that also contain miR-455 binding sites. The 3′UTR of potential targets were cloned into pmiR-GLO and luciferase assays performed. The κB-luc reporter plasmid was used to examine the effect of miR-455 on IL-1β-induced NFκB signalling.

Results: Whole genome array and bioinformatics analyses have identified a number of potential targets of miR-455, including Sirt1. Sirt1 mRNA is regulated across chondrogenesis inversely ot miR-455. Sirt1 mRNA levels decrease when miR-455 is overexpressed and increase when miR-455 is inhibited. Sirt1 also contains a number of miR-455 binding sites. Luciferase assays confirmed that Sirt1 is a direct target of miR-455. A protein substrate of Sirt1 is NFκB/p65. We therefore used an NFκB -responsive reporter to examine the effect of miR-455 on IL-1β signalling through this pathway. Both miR-455 and a Sirtuin inhibitor, nicotinamide, decreased IL1-β signalling. We are currently looking at the effect of miR-455 on the acetylation of Sirt1 target proteins.

Conclusions: We have used bioinformatic analyses of mRNA/miRNA expression datasets to identify a number of genes that are direct targets of miR-455, including Sirt1. We have validated Sirt1 as a miR-455 target. We have also shown that miR-455 can regulate IL1 signalling through NFκB which is potentially due to altered acetylation of Sirt1 substrates, including p65. This represents an important control mechanism for Sirt1, a factor implicated in osteoarthritis.

OA: Ligament/Meniscus/Tendon/Muscle

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DISRUPTIVE PATHOLOGY RATHER THAN DEGENERATIVE OR DISCRETE TEAR ARE ASSOCIATED WITH INCREASING BONE MARROW LESION VOLUME AND A PROXY FOR TOTAL KNEE ARTHROPLASTY: LONGITUDINAL ANALYSIS FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: Meniscal pathology is associated with osteoarthritis (OA) progression including increased bone marrow lesions (BMLs). However, no study has accounted for the different types of meniscal pathology and its association with BMLs or total knee arthroplasty (TKA). The aim of this study was to explore the association of different types of knee meniscal pathology with BML volume, change in BML volume over 2 years, and a proxy for TKA according to a modified Escobar algorithm.

Methods: We selected a convenience sample of the Osteoarthritis Initiative (OAI) who had symptomatic knee OA and complete data for the OAI Bone Ancillary Project. The right knee was selected as the index knee unless there was a contraindication for magnetic resonance (MR) imaging. A single experienced fellowship trained musculoskeletal radiologist reviewed the 24-month OAI MR images for meniscal pathology by location (i.e., anterior, body, and posterior horn) within the medial and lateral menisci using a modified International Society of Arthroscopy, Knee Surgery, and Orthopaedic Sports Medicine (ISAKOS) meniscal tear classification system. For analyses, we reclassified the 10 original ISAKOS categories into 5 categories: normal, degenerative signal, morphological deformity, any tear (i.e., horizontal, horizontal flap, vertical-longitudinal, radial, radial-longitudinal, complex tear), and maceration. Total number of regions affected by meniscal pathology (0-6) was calculated by counting the number of regions in medial and lateral menisci that had pathologic findings. BML volume assessment was performed using a semi-automated segmentation method at 24 and 48 month visits. A CART regression was performed to identify the meaningful abnormal BML volume cut-off value ($1cm^3$) using medial joint space narrowing progression as outcome. We categorized the 24-month BML volume into 3 categories: 1) no meaningful BML volume (< $1cm^3$), 2) >= 1 cm^3 and below median value ($2.15cm^3$) and 3) above median value of meaningful BML volume. The change in BML volume was categorized to 4 groups: 1) no meaningful BML volume (<$1cm^3$) at both time points, 2) lowest quartile of meaningful BML volume change (BML volume >= 1.00 cm^3 at both times & BML volume change <=-0.75 cm^3), 3) middle 2 quartile of the BML volume change (BML volume >= 1.00 cm^3 at both times & BML volume change >-0.75 cm^3&<=1.00 cm^3), 4) highest quartile of the BML volume change (BML volume >= 1.00 cm^3 at both times & BML volume change >1.00 cm^3). We categorized the proxy for TKA into appropriate and non-appropriate based on the algorithm developed by Escobar et al and adapted to OAI by Riddle et al. To replicate prior studies, we explored whether the presence or absence of any meniscal pathology was associated with BMLs and a proxy for TKA. Logistic regression (ordinal for Table 1 and binary for Table 2) was performed to determine the association of baseline meniscal pathology with BML volume, change in BML volume and a proxy for TKA. All models were adjusted for age, sex and body mass index (BMI).

Results: 400 participants were included in the analysis with mean age of 63 (9.2) years, 53% male, BMI 29.6 (4.6) kg/m^2, 71% KL grade >=2, and with 86% having any type of meniscal pathology. Tables below provide the associations between meniscal pathology and BML volume (Table 1), BML volume change (Table 1) and proxy for TKA (Table 2). There was a significant association between any meniscal pathology with BML volume (OR:3.87) and change in BML volume (OR:2.32) but not with proxy for TKA. Having more number of regions of the menisci affected with pathology was associated with greater BML volume, change in BML volume, and proxy for TKA than those with a normal meniscus. Among the types of meniscal pathology, only morphological deformity and maceration were associated with BML volume, change in BML volume, and proxy for TKA. Removing surgery or injury cases did not change our results.

Conclusions: Among the five categories of meniscal pathologies, disruptive pathology rather than degenerative or discrete tear was associated with structural changes and a later clinical state that is proxy for TKA. This suggests that pathologies that impair normal load distribution properties of meniscus can cause damage to the knee joint.

	Cross-sectional BML volume categories	**Longitudinal** BML volume change categories
Menisci	Multivariable* OR (95% CI)	Multivariable* OR (95% CI)
Any Pathology (n=332)	**3.87(1.34,11.23)**	**2.32(1.04,5.21)**
Normal Menisci (n = 55)	Reference	Reference
Number of Regions Affected		
0 (n=55)	Reference	Reference
1 (n=52)	0.62(0.13,2.95)	0.49(0.14,1.62)
2 (n=87)	**3.67(1.17,11.52)**	**2.56(1.05,6.22)**
3 (n=68)	**5.50(1.73,17.45)**	2.42(0.97,6.06)
4 (n=32)	**4.44(1.24,15.88)**	**3.60(1.28,10.14)**
5 (n=48)	**3.47(1.02,11.79)**	2.58(0.98,6.80)
6 (n=44)	**10.13(3.07,33.27)**	**4.67(1.81,12.02)**
Maceration: Number of Regions Affected**		
0 (n=309)	Reference	Reference
1 (n=37)	**2.92(1.40,6.07)**	1.67(0.84,3.32)
2 (n=22)	**7.91(3.06,20.43)**	**3.22(1.44,7.21)**
3 and above (n=18)	**17.11(6.16,47.56)**	**8.25(3.44,19.77)**
Type of Pathology**		
Degeneration (n=212)	1.21 (0.73,2.02)	1.16(0.74,1.82)
Morphological Deformity (n=117)	**2.47(1.49,4.10)**	**2.18(1.38,3.47)**
Maceration (n=77)	**5.68(3.27,9.86)**	**3.01(1.83,4.94)**
Any Discrete Tear (n=183)	0.97(0.59,1.61)	1.20(0.79,1.80)

* Ordinal regression models were used and adjusted for age, gender and BMI

**Types of pathology were further adjusted for each other in multivariable analysis